# Mer-f3, 12-Hydroxy-ovalicin, Produced by *Metarrhizium* sp. f3

Sir:

In the course of our screening program for new antitumor antibiotics, Mer-f3 (**1**), a new ovalicin related compound (Fig. 1), was found in the culture filtrate of *Metarrhizium* sp. f3 which was isolated from a soil sample. In this paper, we report the production, isolation, physico-chemical properties, biological activities and structure of Mer-f3.

The Mer-f3-producing strain, f3, was isolated from a soil sample collected at Kanagawa prefecture, Japan, and was identified as *Metarrhizium* sp. by morphological characteristics. It was deposited at the National Institute of Bioscience and Human-Technology, Ibaraki Prefecture, Japan with accession number FERM P-15860. The strain was cultured at 27°C for 4 days on a rotary shaker (220 rpm) in 500 ml Erlenmeyer flasks containing 50 ml of a medium composed of potato starch 2%, glucose 1%, soy bean meal 2%, $KH_2PO_4$ 0.1% and $MgSO_4 \cdot 7H_2O$ 0.005%.

The culture broth (4.6 liters) was centrifuged at 4000 rpm for 20 minutes to obtain culture supernatant, and was extracted with equal volume of EtOAc. The extract was dried *in vacuo* to give an oily substance (*ca.* 820 mg). The crude material was applied on a silica gel column and the active compound was eluted with $CHCl_3$-MeOH (50:1). The active fractions were collected and concentrated under reduced pressure to yield a yellow powder (157 mg). This powder was purified by a LH-20 chromatography using MeOH to provide 10 mg of pure Mer-f3 (**1**) as a colorless oil.

Physico-chemical properties of **1** are summarized in Table 1. The antibiotic was easily soluble in $CHCl_3$, MeOH, DMSO and hardly soluble in water. It gave positive color reaction to molybdophosphoric acid, 2,4-dinitrophenylhydrazine and anisaldehyde-$H_2SO_4$ reagents and negative to ninhydrin and Rydon-Smith reagents. The UV spectrum had an end absorption and IR spectrum suggested the presence of hydroxy group (3470 $cm^{-1}$) and carbonyl group (1739 $cm^{-1}$).

The molecular formula of **1** was determined to be $C_{16}H_{24}O_6$ base on HRFAB-MS data and was supported by $^1H$ and $^{13}C$ NMR spectral data (Table 1 and 2). All one bond connections between $^1H$ and $^{13}C$ were elucidated by the $^1H$-$^{13}C$ COSY and DEPT experiments. The NMR data and the molecular formula indicated the presence of three methyls, five methylenes, two $sp^3$ methines and one $sp^2$ methine, four quaternary carbons, one carbonyl carbon and two hydroxy groups. The above mentioned physico-chemical properties and NMR data were similar to those of ovalicin (**2**)[1,2]. The direct comparison of $^{13}C$ NMR revealed the following differences (Table 2). One methyl signal (C-12, $\delta$25.4) in **2** was replaced by oxygen-bearing methylene signal (C-12, $\delta$68.2) in **1**. Other signals of **1** coincided with **2**, suggesting the common ovalicin skeleton. All the assignments of protons and carbons of **1** were confirmed by $^1H$-$^1H$

Fig. 1. Structure of Mer-f3, and related compounds.

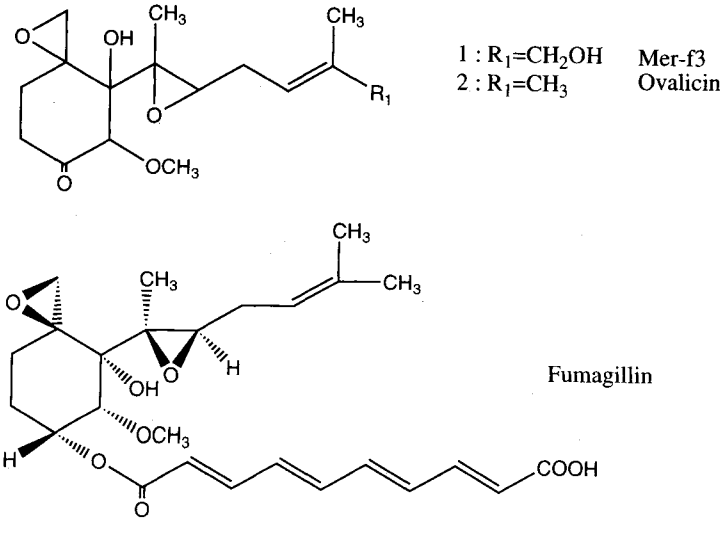

Table 1. Physico-chemical properties of Mer-f3.

| | |
|---|---|
| Appearance | colorless oil |
| Nature | neutral |
| Molecular formula | $C_{16}H_{24}O_6$ |
| FAB-MS (m/z) | 335 $(M+Na)^+$ |
| | 311 $(M-H)^-$ |
| HRFAB-MS (m/z) | |
| Calcd: | 335.1471 ($C_{16}H_{24}O_6Na$) |
| Found: | 335.1465 $(M+Na)^+$ |
| UV $\lambda$max ($\varepsilon$) in MeOH | End absorption |
| IR $\upsilon$max (neat) $cm^{-1}$ | 3470, 2930, 1730, 1440, 1380, |
| | 1110, 1030, 1000 |
| $[\alpha]_D^{29}$ | -46° (*c* 0.5, MeOH) |
| Rf | 0.16[a] |
| | 0.21[b] |

a: Silica gel TLC (Merck Art. No. 105715) $CHCl_3$-MeOH=10:1

b: Silica gel TLC (Merck Art. No. 105715) toluene-EtOAc=1:1

Table 2. NMR data of Mer-f3 and ovalicin.

| | Mer-f3 in $CDCl_3$ | | Ovalicin in $CDCl_3$ [2] |
|---|---|---|---|
| Position | $^{13}C$(Multiplicity) | $^1H$(Multiplicity, *J* value, Hz) | $^{13}C$(Multiplicity) |
| 1 | 78.5(s) | | 78.2(s) |
| 2 | 86.0(d) | 4.24(1H, s) | 85.9(d) |
| 3 | 206.5(s) | | 206.2(s) |
| 4 | 36.6(t) | 2.50(1H, m), 2.68(1H, m) | 36.4(t) |
| 5 | 30.2(t) | 1.45(1H, m), 2.62(1H, m) | 30.0(t) |
| 6 | 60.5(s) | | 60.2(s) |
| 7 | 60.3(s) | | 60.0(s) |
| 8 | 56.5(d) | 2.95(1H, t, *J*=6.60) | 56.5(d) |
| 9 | 26.5(t) | 2.28(1H, m), 2.41(1H, m) | 26.8(t) |
| 10 | 119.0(d) | 5.52(1H, td, *J*=7.33, 1.46) | 117.8(d) |
| 11 | 138.3(s) | | 135.0(s) |
| 12 | 68.2(t) | 4.06(2H, d, *J*=4.76) | 25.4(d) |
| 13 | 13.9(q) | 1.71(3H, s) | 17.7(q) |
| 14 | 51.4(t) | 3.05(1H, d, *J*=4.03), 2.75(1H, d, *J*=4.03) | 51.0(t) |
| 15 | 14.4(q) | 1.38(3H, s) | 14.1(q) |
| OMe | 59.2(q) | 3.57(3H, s) | 59.6(q) |
| 1-OH | | 3.16(1H, s) | |
| 12-OH | | 1.68(br. s) | |

Fig. 2. $^{1}H$-$^{1}H$ coupling by COSY and $^{1}H$-$^{13}C$ long range coupling by HMBC.

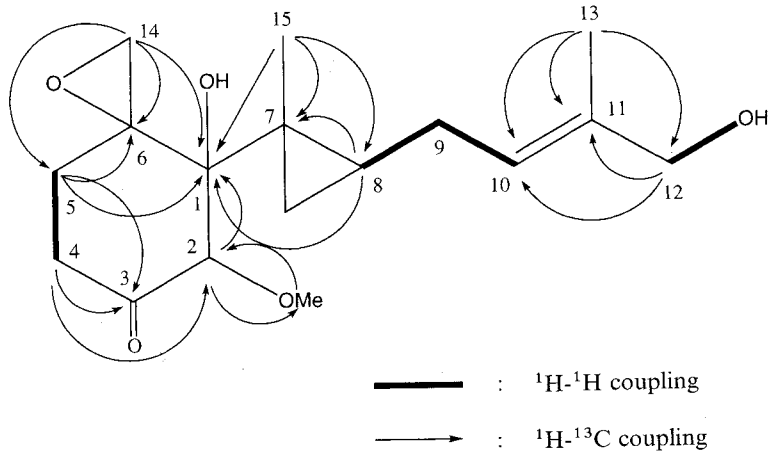


Table 3. Cytotoxicity of Mer-f3 against human tumor cells.

| Cells | $IC_{50}$ (M) |
|---|---|
| K562 | $3.8 \times 10^{-5}$ |
| HT29 | $1.5 \times 10^{-4}$ |
| MCF7 | $2.2 \times 10^{-4}$ |
| HT1080 | $1.2 \times 10^{-8}$ |

Table 4. Inhibitory effects on Mer-f3 against MLCR, growth of L-1210 cells.

| Compounds | MLCR (IC50(M)) | L-1210 (IC50 (M)) |
|---|---|---|
| Mer-f3 | $1.0 \times 10^{-9}$ | $>10^{-4}$ |
| FK506 | $1.3 \times 10^{-8}$ | $>10^{-4}$ |
| Cyclosporin | $1.1 \times 10^{-7}$ | $8 \times 10^{-6}$ |

COSY and HMBC experiments as shown in Fig. 2. On the bases of the above data, the planar structure of Mer-f3 (**1**) was established to be 12-hydroxy-ovalicin (Fig. 1). The relative and absolute structure of **1** are now in progress.

The cytotoxic activities of Mer-f3 against human tumor cell lines including chronic myelogenous leukemia K562, colon carcinoma HT29, breast carcinoma MCF7 and fibrosarcoma HT1080 were assessed. These cells were cultured for 3 days and the cell growth was measured by the WST-1 method[3]. As shown in Table 3, Mer-f3 showed a potent inhibitory activity against HT1080 cells, while it showed relatively weak ones against K562, HT29 and MCF7 cells.

The inhibitory activity of Mer-f3 against human umbilical vein endothelial cells (HUVEC) was also examined. HUVEC were cultured in MCDB-131 medium supplemented with 10% FCS and 10 ng/ml of bFGF for 5 days, and the cell growth was measured by counting live cells with trypan blue dying. Mer-f3 ($IC_{50} = 3.5 \times 10^{-9}$ M) and fumagillin ($IC_{50} = 2.6 \times 10^{-9}$ M) showed a potent inhibitory activity against HUVEC growth to the same extent.

Since ovalicin, structurally similar to Mer-f3, is known to have a point immunosuppressive activity as well as FK506 and cyclosporin[4], we examined the effects of Mer-f3 on a mixed lymphocyte culture reaction (MLCR) and growth of L-1210 mouse leukemia cells. The MLCR was induced as described previously[5]. Briefly, responder spleen cells taken from C3H/He mice were mixed with stimulator cells taken from Balb/c mice. The stimulator cells were treated with 5 $\mu$g/ml of mitomycin C for 30 minutes and washed the drug out. The mixed cells were cultured for 3 days and [$^{3}H$]TdR was added to the culture 16 hours before cell harvest. MLCR was determined by measuring the incorporation of [$^{3}H$]TdR into the cultured cells. As shown in Table 4, Mer-f3 showed the most potent inhibitory activity against MLCR among the three compounds examined; the inhibitory activity of Mer-f3 was similar to that of ovalicin[6]. Mer-f3 and

FK506, unlike cyclosporin ($IC_{50}=8\times10^{-6}$ M), had no inhibitory activity against L-1210 cell growth even at a concentration of $10^{-4}$ M.

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